

using injection, by aerosol, sublingually, topically (including to a mucosal surface), and by gene therapy (for example, by injection of the gene encoding the immunotherapeutic into muscle or skin where it is transiently expressed for a time sufficient to induce tolerance).

5 This method and the criteria for identifying and altering allergens can be used to design useful proteins (including nucleotide molecules encoding the proteins) for use in immunotherapy, to make a vaccine and to genetically engineer organisms such as plants and animals which then produce proteins with less likelihood of eliciting an IgE response. Techniques for engineering 10 plants and animals are well known. Based on the information obtained using the method described in the examples, one can engineer plants or animals to cause either site specific mutations in the gene encoding the protein(s) of interest, or to knock out the gene and then insert the gene encoding the modified protein.

15 **Brief Description of the Drawings**

Figure 1 shows an example of how IgE binding epitopes were mapped to a specific amino acid sequence (SEQ ID NO:2) on the Ara h 1 allergen.

Figure 2 shows how IgE binding epitopes were mapped to a specific amino acid sequence (SEQ ID NO:4) on the Ara h 2 allergen.

20 Figure 3 shows how IgE binding epitopes were mapped to a specific amino acid sequence (SEQ ID NO:6) on the Ara h 3 allergen.

Figure 4 shows how amino acids critical to IgE binding were identified.

Figure 5A shows the location of altered residues within the Ara h 2 amino acid sequence (SEQ ID NO 4).

25 Figure 5B shows the effect the modified Ara h 2 protein has on IgE binding.

Figure 5C shows the effect the modified Ara h 2 protein has on IgG binding.



Table 1. Ara h I IgE Binding Epitopes

EPITOPE	AA SEQUENCE	POSITION
1 (nucleotides 25-34 of SEQ ID NO:2)	<u>AKSSPYQKKT</u>	25-34
2 (nucleotides 48-57 of SEQ ID NO:2)	<u>QEPDDLQKQA</u>	48-57
3 (nucleotides 65-74 of SEQ ID NO:2)	<u>LEYDPLRVYD</u>	65-74
4 (nucleotides 89-98 of SEQ ID NO:2)	<u>GERTRGRQPG</u>	89-98
5 (nucleotides 97-106 of SEQ ID NO:2)	<u>PGDYDDDRRQ</u>	97-106
6 (nucleotides 107-116 of SEQ ID NO:2)	<u>PRREEGGRWG</u>	107-116
7 (nucleotides 123-132 of SEQ ID NO:2)	<u>REREEDWRQP</u>	123-132
8 (nucleotides 134-143 of SEQ ID NO:2)	<u>EDWRRPSHQQ</u>	134-143
9 (nucleotides 143-152 of SEQ ID NO:2)	<u>QPRKIRPEGR</u>	143-152
10 (nucleotides 294-303 of SEQ ID NO:2)	<u>TPGQFEDFFP</u>	294-303
11 (nucleotides 311-320 of SEQ ID NO:2)	<u>SYLQEFSRNT</u>	311-320
12 (nucleotides 325-334 of SEQ ID NO:2)	<u>FNAEFNEIRR</u>	325-334
13 (nucleotides 344-353 of SEQ ID NO:2)	<u>EQEERGQRRW</u>	344-353
14 (nucleotides 393-402 of SEQ ID NO:2)	<u>DITNPINLRE</u>	393-402
15 (nucleotides 409-418 of SEQ ID NO:2)	<u>NNFGKLFEVK</u>	409-418
16 (nucleotides 461-470 of SEQ ID NO:2)	<u>GTGNLELVAV</u>	461-470
17 (nucleotides 498-507 of SEQ ID NO:2)	<u>RRYTARLKEG</u>	498-507
18 (nucleotides 525-534 of SEQ ID NO:2)	<u>ELHLLGFGIN</u>	525-534
19 (nucleotides 539-548 of SEQ ID NO:2)	<u>HRIFLAGDKD</u>	539-548
20 (nucleotides 551-560 of SEQ ID NO:2)	<u>IDQIEKQAKD</u>	551-560
21 (nucleotides 559-568 of SEQ ID NO:2)	<u>KDLAFFPGSGE</u>	559-568
22 (nucleotides 578-587 of SEQ ID NO:2)	<u>KESHFVSARP</u>	578-587
23 (nucleotides 597-606 of SEQ ID NO:2)	<u>PEKESPEKED</u>	597-606

The underlined portions of each peptide are the smallest IgE binding sequences as determined by this analysis. All of these sequences can be found in SEQ ID NO 2.



Table 2. Ara h 2 IgE Binding Epitopes²³

EPITOPE	AA SEQUENCE	POSITION
1 (nucleotides 15-24 of SEQ ID NO:4)	<u>HASARQQWEL</u>	15-24
2 (nucleotides 21-30 of SEQ ID NO:4)	<u>QWELQGDRRC</u>	21-30
3 (nucleotides 27-36 of SEQ ID NO:4)	<u>DRRCOSOLER</u>	27-36
4 (nucleotides 39-48 of SEQ ID NO:4)	<u>LRPCEQHLMQ</u>	39-48
5 (nucleotides 49-58 of SEQ ID NO:4)	<u>KIQRDEDSYE</u>	49-58
6 (nucleotides 57-66 of SEQ ID NO:4)	<u>YERDPYSPSQ</u>	57-66
7 (nucleotides 65-74 of SEQ ID NO:4)	<u>SQDPYSPSPY</u>	65-74
8 (nucleotides 115-124 of SEQ ID NO:4)	<u>DRLQGROQQEQ</u>	115-124
9 (nucleotides 127-136 of SEQ ID NO:4)	<u>KRELRNLPQQ</u>	127-136
10 (nucleotides 143-152 of SEQ ID NO:4)	<u>QRCLDLDVESG</u>	143-152

The underlined portions of each peptide are the smallest IgE binding sequences as determined by this analysis. All of these sequences can be found in SEQ ID NO 4.

Table 3. Ara h 3 IgE Binding Epitopes

EPITOPE	AA SEQUENCE	POSITION
1 (nucleotides 33-47 of SEQ ID NO:6)	<u>IETWNPNNNQEFECAG</u>	33-47
2 (nucleotides 240-254 of SEQ ID NO:6)	<u>GNIFSGFTPEFLEQA</u>	240-254
3 (nucleotides 279-293 of SEQ ID NO:6)	<u>VTVRGGLRILSPDRK</u>	279-293
4 (nucleotides 303-317 of SEQ ID NO:6)	<u>DEDEYEYDEEDRRRG</u>	303-317

The underlined portions of each peptide are the smallest IgE binding sequences as determined by this analysis. All of these sequences can be found in SEQ ID NO 6.



Table 4: Amino Acids Critical to IgE Binding of Ara h 1

EPITOPE	AA SEQUENCE	POSITION
1 (nucleotides 25-34 of SEQ ID NO:2)	A K <u>S</u> S P <u>Y</u> Q K <u>K</u> T	25-34
2 (nucleotides 48-57 of SEQ ID NO:2)	Q E <u>P</u> D <u>D</u> L <u>K</u> Q <u>K</u> A	48-57
3 (nucleotides 65-74 of SEQ ID NO:2)	L E <u>Y</u> D P <u>R</u> L <u>V</u> Y D	65-74
4 (nucleotides 89-98 of SEQ ID NO:2)	G E <u>R</u> T <u>R</u> G <u>R</u> Q <u>P</u> G	89-98
5 (nucleotides 97-106 of SEQ ID NO:2)	P <u>G</u> D <u>Y</u> D <u>D</u> D R <u>R</u> Q	97-106
6 (nucleotides 107-116 of SEQ ID NO:2)	P <u>R</u> R <u>E</u> E <u>G</u> G <u>R</u> W <u>G</u>	107-116
7 (nucleotides 123-132 of SEQ ID NO:2)	R <u>E</u> R <u>E</u> E <u>D</u> W R <u>Q</u> P	123-132
8 (nucleotides 134-143 of SEQ ID NO:2)	E <u>D</u> W <u>R</u> R <u>P</u> S <u>H</u> Q <u>Q</u>	134-143
9 (nucleotides 143-152 of SEQ ID NO:2)	Q P <u>R</u> K <u>I</u> R <u>P</u> E <u>G</u> R	143-152
10 (nucleotides 294-303 of SEQ ID NO:2)	T P <u>G</u> Q <u>F</u> E <u>D</u> F F <u>P</u>	294-303
11 (nucleotides 311-320 of SEQ ID NO:2)	S Y <u>L</u> Q <u>E</u> E <u>F</u> S <u>R</u> N <u>T</u>	311-320
12 (nucleotides 325-334 of SEQ ID NO:2)	F <u>N</u> A <u>E</u> F <u>N</u> E <u>I</u> R <u>R</u>	325-334
13 (nucleotides 344-353 of SEQ ID NO:2)	E <u>Q</u> E <u>E</u> R <u>G</u> Q <u>R</u> R <u>W</u>	344-353
14 (nucleotides 393-402 of SEQ ID NO:2)	D I <u>T</u> N <u>P</u> I <u>N</u> L <u>R</u> E	393-402
15 (nucleotides 409-418 of SEQ ID NO:2)	N <u>N</u> F <u>G</u> K <u>L</u> F <u>E</u> V <u>K</u>	409-418
17 (nucleotides 498-507 of SEQ ID NO:2)	R R <u>Y</u> T <u>A</u> R <u>L</u> K <u>E</u> G	498-507
18 (nucleotides 525-534 of SEQ ID NO:2)	E <u>L</u> <u>H</u> <u>L</u> <u>L</u> <u>G</u> <u>F</u> G <u>I</u> N	525-534
19 (nucleotides 539-548 of SEQ ID NO:2)	H <u>R</u> I <u>F</u> L <u>A</u> G <u>D</u> K D	539-548
20 (nucleotides 551-560 of SEQ ID NO:2)	I <u>D</u> Q <u>I</u> E <u>K</u> Q <u>A</u> K <u>D</u>	551-560
21 (nucleotides 559-568 of SEQ ID NO:2)	K <u>D</u> L <u>A</u> F <u>P</u> G <u>S</u> G <u>E</u>	559-568
22 (nucleotides 578-587 of SEQ ID NO:2)	K <u>E</u> S <u>H</u> F <u>V</u> S A <u>R</u> P	578-587

Note. The Ara h 1 IgE binding epitopes are indicated as the single letter amino acid code. The position of each peptide with respect to the Ara h 1 protein is indicated in the right hand column. The amino acids that, when altered, lead to loss of IgE binding are shown as the bold, underlined residues. Epitopes 16 and 23 were not included in this study because they were recognized by a single patient who was no longer available to the study. All of these sequences can be found in SEQ ID NO 2.



Table 5. Amino Acids Critical to IgE Binding of Ara h 2

EPITOPE	AA SEQUENCE	POSITION
1 (nucleotides 15-24 of SEQ ID NO:4)	HASAR<u>QQ</u>WEL	15-24
2 (nucleotides 21-30 of SEQ ID NO:4)	<u>QWEL</u>QGDRRC	21-30
3 (nucleotides 27-36 of SEQ ID NO:4)	DRRC<u>QSQLER</u>	27-36
4 (nucleotides 39-48 of SEQ ID NO:4)	<u>LRPCEQHLMQ</u>	39-48
5 (nucleotides 49-58 of SEQ ID NO:4)	<u>KIQR</u>DEDSYE	49-58
6 (nucleotides 57-66 of SEQ ID NO:4)	<u>YERDPY</u>SPSQ	57-66
7 (nucleotides 65-74 of SEQ ID NO:4)	<u>SQDPY</u>SPSPY	65-74
8 (nucleotides 115-124 of SEQ ID NO:4)	DRL<u>QGR</u>QQEQ	115-124
9 (nucleotides 127-136 of SEQ ID NO:4)	<u>KREL</u>RNLPQQ	127-136
10 (nucleotides 143-152 of SEQ ID NO:4)	QR<u>CDLDV</u>ESG	143-152

Note. The Ara h 2 IgE binding epitopes are indicated as the single letter amino acid code. The position of each peptide with respect to the Ara h 2 protein is indicated in the right hand column. The amino acids that, when altered, lead to loss of IgE binding are shown as the bold, underlined residues. All of these sequences can be found in SEQ ID NO 4.

Table 6. Amino Acids Critical to IgE-Binding of Ara h 3.

EPITOPE	AA SEQUENCE	POSITION
1 (nucleotides 33-47 of SEQ ID NO:6)	IETWN<u>PNN</u>QEFCAG	33-47
2 (nucleotides 240-254 of SEQ ID NO:6)	<u>GNIFSGFT</u>PEFLEQA	240-254
3 (nucleotides 279-293 of SEQ ID NO:6)	VTVRGG<u>L</u>RILSPDRK	279-293
4 (nucleotides 303-317 of SEQ ID NO:6)	DEDEY<u>EYDEED</u>RRRG	303-317

Note. The Ara h 3 IgE binding epitopes are indicated as the single letter amino acid code. The position of each peptide with respect to the Ara h 3 protein is indicated in the right hand column. The amino acids that, when altered, lead to loss of IgE binding are shown as the bold, underlined. All of these sequences can be found in SEQ ID NO 6.